UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION PREVENTION

November 5, 2014

Rhonda Jones
Science and Regulatory Consultants
P.O. Box 1014
Columbia City, IN 46725

Subject: Protocol: Microban® Firebird 127 Disinfectant
EPA Registration File Symbol: 42812-PA-3

Dear Ms. Jones:

The Agency has completed its review of the above mentioned Protocol and has attached the efficacy review for your reference.

Should you have any questions or comments concerning this letter, please contact me via electronic mail: chao.julie@epa.gov or by telephone (703) 308-6416 or Stacey Grigsby via electronic mail at grigsby.stacey@epa.gov or by telephone at (703) 305-6440.

Sincerely,

Stacey Grigsby

/Julie Chao
Acting Product Manager (34)
Regulatory Management Branch II
Antimicrobials Division (7510P)

Enclosure: 10/30/14 efficacy review

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

October 30, 2014

MEMORANDUM

Subject: Protocol Review for 42182PA3 (Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use); DB Barcode: D422195.

From: Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry
Team Leader
Product Science Branch
Antimicrobials Division (7510P)

To: Stacey Grigsby
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Microban International, Ltd.
11400 Vanstory Drive
Huntersville, NC 28078.

I. BACKGROUND

Microban International, Ltd. intends to conduct an analysis of dried product efficacy, following EPA Protocol # 01-1A and guidance received from the Antimicrobial Division's Efficacy Team. Through the current submission, the registrant is submitting a bacterial efficacy protocol to support residual self-sanitization/disinfection claims for hospital use for a liquid/spray disinfectant product (Microban Firebird 127 Disinfectant). Protocol was developed by Antimicrobial Test Laboratories (ATL), located at 1304 W. Industrial Blvd., Round Rock, Texas 78681.

This data package is identified as D422195 contained a letter from the applicant's representative (dated July 15, 2014), and one protocols (MRID no. 494316-01).

II. BRIEF DESCRIPTION OF THE PROTOCOL

MRID 494316-01

Title:
Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use

Purpose:
The purpose of this study is to document the residual activity of the test substance against the test systems (microorganisms) under the test parameters specified in this protocol

Active Ingredient Concentration

Method References:
EPA Protocol # 01-1A. Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces.

Test System (Microorganism):

Staphylococcus aureus ATCC 6538
Enterobacter aerogenes ATCC 13048
Pseudomonas aeruginosa ATCC 15442

Additional Bacteria: {Insert additional strains (e.g. E. coli, MRSA)}

Required bacteria will be tested on 3 lots per organism. Additional bacteria will be tested on 2 lots per organism.

Study Parameters

Residual Self-Sanitizing / Self-Disinfecting Efficacy

Contact Time - {Insert exposure period (e.g. 5mins, 10mins, 1 hour).

For Residual Sanitizer claims, a 99.9% reduction must be demonstrated in ≤ 5 minutes ± 5 seconds.

For Residual Disinfection claims, a 99.999% reduction must be demonstrated in ≤ 10 minutes ± 5 seconds

Continuous Reduction Period - {Insert time period (e.g. 12 hours, 24 hours). The following procedure is based on a 24 hour continuous reduction period thus the procedure must be completed in >24 hours but no longer than 48 hours. If shorter or longer continuous reduction claims are desired, then the Wear, Abrasion, and Re-inoculation frequencies and the laboratory duration of the procedure must be adjusted ratiometrically. The period must not be less than 4 hours. For example to support a 12 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be reduced by 50% and the study must be completed in >12 hours but no longer than 24 hours. For example to support a 48 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be doubled and the study must be completed in >48 hours but no longer than 96 hours.}

Abrasion Control Replicates - 4 per test microorganism
Non-Abrasion Control Replicates - 4 per test microorganism
Test Surface Replicates - 4 per test substance, per test microorganism
Neutralization Controls - 2 per test substance, per test microorganism inoculum level

Exposure of Test Carriers to Test Substance

- Four test carriers (per lot, per microorganism) are treated by spray application with the test substance. Each carrier is treated according to the study sponsor's instructions.
- After treatment, the test substance on the carriers is allowed to dry at room temperature and 45-55% relative humidity with the lids ajar for up to 1 hour such that the first Wear cycle begins no later than no longer than 1 hour after treatment of the carrier.

Abrasions and Re-inoculations

- Test Carriers and Abrasion Control Carriers undergo a wear and re-inoculation regimen including a series of at least 12 wear cycles and 12 re-inoculation cycles to support a 24 hour continuous reduction claim. The Non-Abrasion Control Carriers do not undergo the wear cycling. The number, duration, and order of abrasions may be ratiometrically modified by the Study Sponsor to support the desired claim as described above. This step is performed at room temperature. The table below summarizes the manipulations of all carriers in the study.
- Abrasions are conducted between 45-55% relative humidity (RH). Temperature and room humidity measurements are taken and recorded periodically throughout the abrasion process.
- The weight of the fully assembled abrasion boats are recorded prior to initiation of the wear and reinoculation regimen and must equal 1084 ± 1.0g
- The abrasion tester is set to a speed of 2.25 to 2.5 for a total surface contact time of approximately 8-10 seconds, for one complete abrasion cycle. Each abrasion cycle in this test equals four (4) passes, one pass to the left and one return pass to the right followed by another pass to the left and another return pass to the right.
- All surfaces in contact with carriers on the abrasion tester are decontaminated with ethanol and allowed to dry completely between each set of surface wears to prevent carryover contamination.
- The foam liner and cotton cloths on the abrasion tester are replaced between each set of abrasion.

- After each complete set of abrasions are conducted (all control and test carriers abraded), the carriers are allowed to sit undisturbed for at least 15 minutes.
- Control and test carriers are then re-inoculated with 0.010ml of the re-inoculation culture and spread within 1/8 inch of the surface edge, and then allowed to dry at ambient temperature for a minimum of 30 minutes or until completely dry prior to initiation of the next set of abrasions.
- Cotton cloths used as part of wet abrasions are prepared individually prior to each wet abrasion cycle by spraying the cloth with sterile reverse osmosis water using a sanitized Preval sprayer, from a distance of 75±1cm for no more than 1 second and used immediately.

Procedure Timeline (Hours)	Abrasion/Re-inoculation Procedure	Target CFU/Carrier
0	Initial inoculation of Test and Control Carriers	10^6
1	Test Substance Application and Drying	
1-24	Dry Abrasion (wear #1)	10^4 with each reinoculation
	Re-inoculation (1)*	
	Wet Abrasion (wear #2)	
	Re-inoculation (2)*	
	Dry Abrasion (wear #3)	
	Re-inoculation (3)*	
	Wet Abrasion (wear #4)	
	Re-inoculation (4)*	
	Dry Abrasion (wear #5)	
	Re-inoculation (5)*	
	Wet Abrasion (wear #6)	
	Re-inoculation (6)*	
	Dry Abrasion (wear #7)	
	Re-inoculation (7)*	
	Wet Abrasion (wear #8)	
	Re-inoculation (8)*	
	Dry Abrasion (wear #9)	
	Re-inoculation (9)*	
	Wet Abrasion (wear #10)	
	Re-inoculation (10)*	
	Dry Abrasion (wear #11)	
	Re-inoculation (11)*	
	Wet Abrasions (wear #12)	
≥24-48	Determination of Residual activity	10^6

Success Criteria:

• The experimental success (controls) criteria follow:

1. In the Neutralization Control, test substance treated carrier counts must be within 0.50 $\log_{10}$ of the control treated carrier counts.
2. The media sterility controls are negative for growth.
3. The purity "isolation streaks" demonstrate a pure culture of test microorganism as evidenced by colony morphology.
4. The carrier sterility controls are negative for growth.

5. The soil sterility control is negative for growth.
6. The Initial Inoculation Carrier Control must have a minimum of 1 x 10^6 CPU/carrier.
7. The Re-Inoculation Carrier Control carriers must have a minimum of 1 x 10^4 CPU/carrier.
8. The Final Abrasion Control must have a minimum of 1 x 10^6 CPU/carrier.

- Test substance performance criterion for public health claims:
  - To be defined as a residual disinfectant for healthcare use, product must:
    - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
    - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 5 $\log_{10}$ or 99.999% at a contact time of ≤10 minutes.
  - To be defined as a residual sanitizer for healthcare use, the test product must:
    - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
    - meet the OCSPP 810.2300 requirements for a non-food contact sanitizer, and
    - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 3 $\log_{10}$ or 99.9% at a contact time of ≤5 minutes.

III. CONCLUSION AND COMMENTS

1. The submitted protocol under MRID 494316-01 **is adequate for testing** 24 hours residual bacterial activity of dried chemical on hard non-porous hospital environment surfaces. However, "Initial inoculation of Test and Control Carriers" step cannot precede "Test Substance Application and Drying" step if residual activity of dried chemical is being tested. **Registrant must revise procedure table to reflect "Test Substance Application and Drying" form 0 to 1 hour; followed by "Initial inoculation of Test and Control Carriers"**.

2. Because the protocol is being used to conduct efficacy testing of dried chemical on hospital environmental hard non-porous, **the following claims are not acceptable**:

a. Residual Self-Sanitization of dried chemical; **all references to self-sanitization must be removed**.

b. Additional bacterial residual self-sanitization or self-disinfection of dried chemical; **all references to residual self-sanitization or self-disinfection activity on additional bacteria must be removed**.

c. Continuous bacterial reduction claims exceeding 24 hours for hospital environmental hard non-porous. **All references to continuous residual self-sanitization or self-disinfection exceeding 24 hours must be removed**.

3. It is a reminder that product must be tested at the lower certified limit proposed on the CSF.

4. The potential variability in the method must be addressed prior to data generation. The Agency encourages the testing laboratory to assess the degree and sources of variability introduced by any significant method modification – this information should be supplied to the Agency prior to GLP testing. For example, preliminary runs of the study should be performed to determine the degree of variability associated with control and treated carriers; the number of carriers should be increased if the variability is too high.

5. Identify and use the most recent versions of all standard methods cited in the protocol. Specify the broth media for generating test cultures and the plating medium for recovery of each test microbe [Use the AOAC Use-dilution method for preparation of cultures of *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 10708), or *Staphylococcus aureus* (ATCC 6538).]

6. The study controls must perform according to the criteria detailed in the protocol. If any of the control acceptance criteria are not met, the test may be repeated.

7. Provide a list of any deviation or modification to a standard method.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

July 18, 2014

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

SCIENTIFIC & REGULATORY CONSULTANTS, INC.
MICROBAN PRODUCTS COMPANY
PO.BOX : 1014
COLUMBIA CITY, IN 46725

Report of Analysis for Compliance with PR Notice 11-03

Thank you for your submittal of 16-JUL-14. Our staff has completed a preliminary analysis of the material. The results are provided as follows:

Your submittal was found to be in full compliance with the standards for submission of data contained in PR Notice 11-03. A copy of your bibliography is enclosed, annotated with Master Record ID's (MRIDs) assigned to each document submitted. Please use these numbers in all future references to these documents. Thank you for your cooperation. If you have any questions concerning this data submission, please raise them with the cognizant Product Manager, to whom the data have been released.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

July 18, 2014

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

OPP Decision Number: D-493252
EPA Pre-Application Number: 42182PA3
Subject:Public Health Efficacy Study Protocol
EPA Receipt Date: 16-Jul-2014
EPA Company Number: 42182
Company Name: MICROBAN PRODUCTS COMPANY

RHONDA JONES
SCIENTIFIC & REGULATORY CONSULTANTS, INC.
AGENT FOR: MICROBAN PRODUCTS COMPANY
PO BOX 1014
COLUMBIA CITY, IN 46725

SUBJECT: Receipt of Protocol Subject to Registration Service Fee

Dear Registrant:

The Office of Pesticide Programs has received your action and certification of payment. If you submitted data with this action, the results of the PRN-2011-3 screen will be communicated separately. During the administrative screen, the Office of Pesticide Programs has determined that this Action is subject to a Pesticide Registration Service Fee as defined in the Pesticide Registration Improvement Act.

The Action has been identified as Action Code: A521

APPLICANT-INITIATED 2;PER AD INTERNAL GUIDANCE FOR THE EFFICACY PROTOCOL REVIEW PROCESS;REVIEW OF PUBLIC HEALTH EFFICACY STUDY PROTOCOL WITHIN AD;TIER 1;

No additional payment is due at this time.

If you have any questions, please contact the Pesticide Registration Service Fee Ombudsman at (703) 308-6427.

Sincerely,
Teresa Downs
Front End Processing Staff
Information Technology & Resources Management Division

July 15, 2014

Jacqueline Hardy
U.S. Environmental Protection Agency
Document Processing Desk (PROTOCOL)
Office of Pesticide Programs (7504P)
Room S4900, One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

SUBJECT: Protocol for: Microban® Firebird 127 Disinfectant
(EPA Reg. No. 42182-PA-2)
Registrant Initiated Tier 1 (internal) efficacy study protocol review
PRIA code A521, $2,363 fee with 3 month review time

Dear Jacquie,

On behalf of our client, Microban International Ltd. (Microban), we are submitting an efficacy protocol for Internal Review to support residual self-sanitization/disinfection claims for hospital use for a liquid/spray disinfectant product (Microban® Firebird 127 Disinfectant).
This product is for use on hard, nonporous surfaces. The protocol was developed in accordance with guidance received from EPA through teleconferences and correspondence (attached).

We have provided a sample label to further illustrate the use directions and intended label claims for the Residual Self-Sanitizing/Disinfecting product.

Please contact me at (260) 244-6270 or rjones@srcconsultants.com if questions arise regarding this action.

Sincerely,

Rhonda Jones

Rhonda D. Jones, RM(AAM)
Agent for Microban International, Ltd.
President, Scientific & Regulatory Consultants, Inc.

cc: Neil Snyder, Microban
Gina Sloan, Microban
Ivan Ong, Microban

From: Laniyan, Ibrahim
To: riones@srcconsultants.com
Subject: RE: New Efficacy Protocol Meeting Request
Date: Friday, January 31, 2014 6:02:44 PM

This is the promised table for self-sanitization or self-disinfection on hard non-porous surfaces:

Hours	Minimum CFU/Carrier	Abrasion/Re-inoculation Procedure
0-1.5	$1x10^6$ for Bacteria	Test/Control Substance application and drying
		Inoculation of All Carriers with initial inoculation culture
1.5-24	$1x10^4$ for Bacteria	One Wet and one Dry Abrasions (wear cycle #1)
		Re-inoculation (1)*
		One Wet and one Dry Abrasions (wear cycle #2)
		Re-inoculation (2)*
		One Wet and one Dry Abrasions (wear cycle #3)
		Re-inoculation (3)*
		One Wet and one Dry Abrasions (wear cycle #4)
		Re-inoculation (4)*
		One Wet and one Dry Abrasions (wear cycle #5)
		Re-inoculation (5)*
		One Wet and one Dry Abrasions (wear cycle #6)
		Re-inoculation (6)*
		One Wet and one Dry Abrasions (wear cycle #7)
		Re-inoculation (7)*
		One Wet and one Dry Abrasions (wear cycle #8)
		Re-inoculation (8)*
		One Wet and one Dry Abrasions (wear cycle #9)
		Re-inoculation (9)*
		One Wet and one Dry Abrasions (wear cycle #10)
		Re-inoculation (10)*
		One Wet and one Dry Abrasions (wear cycle #11)
		Re-inoculation (11)*
		One Wet and one Dry Abrasions (wear cycle #12)
= 24	$1x10^6$ for Bacteria	Residual Self-Sanitization or Disinfection Test

- Carriers must undergo a minimum of 24 wears/abrasions organized in cycles for 24 hours residual disinfection claims. Each abrasion equals one pass to the left and one return pass to the right; each cycle is composed of one wet abrasion followed by one dry abrasion (12 "wear/abrasion cycles"). Using Gardco Washability and Wear Tester (Model D10V), set speed at 2.25 to 2.5 for a total surface contact time of approximately 4-5 seconds; weight of the fully assembled abrasion boats must equal 1084±1.0 g.

- The effectiveness of the neutralizer is valid if controls are within $0.5\log_{10}$ of the control (31.6% or greater) for bacteria, fungi and viruses; using a small number of challenge (e.g., less than 200-300).

In your case, the table will be twisted. This table is to guide you.

Thank you

Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
Antimicrobials Division

Office of Pesticide Programs
1200 Pennsylvania Ave., NW (7510P)
Washington, DC 20460
Tel. (703) 308-0124
Fax (703) 308-8481

From: rjones@srcconsultants.com [mailto:rjones@srcconsultants.com]
Sent: Friday, January 31, 2014 5:24 PM
To: Grigsby, Stacey
Cc: Campbell, Jacqueline; 'neil.snyder@microban.com' (neil.snyder@microban.com); Gina Sloan; Pat Quinn (pquinn@theaccordgroup.com); Ivan Ong (Ivan.Ong@Microban.com); Laniyan, Ibrahim
Subject: RE: New Efficacy Protocol Meeting Request
Importance: High

Stacey,

Thank you for all your guidance and help in organizing a very productive meeting yesterday. Please thank all the staff for their time and expertise. It was very enlightening for us.

While we are preparing minutes to share, we wondered if it might be possible to obtain a copy of that Residual Testing Table that Ibrahim mentioned during our meeting. We would like to jump into the lab and try out some of the suggestions asap!

Thanks in advance for sending that over! Have a great weekend (Up to 6 inches of snow expected tonight and 20 inches on Tuesday)!

Rhonda

Rhonda Jones, RM(NRM)
Scientific & Regulatory Consultants, Inc.
PO Box 1014
Columbia City, IN 46725
260-244-6270
Fax: 260-244-6273
E-mail: rjones@srcconsultants.com
Web Site: www.srcconsultants.com

From: Grigsby, Stacey [mailto:Grigsby.Stacey@epa.gov]
Sent: Tuesday, January 28, 2014 3:56 PM
To: rjones@srcconsultants.com
Cc: Campbell, Jacqueline; 'neil.snyder@microban.com' (neil.snyder@microban.com); Gina Sloan; Pat Quinn (pquinn@theaccordgroup.com)
Subject: RE: New Efficacy Protocol Meeting Request

Thanks Rhonda, I'll make sure efficacy has the latest updates.
Have a safe flight here and please give me a call when you've arrived to the lobby.
See you Thursday.

From: rjones@srcconsultants.com [mailto:rjones@srcconsultants.com]

Microban® Firebird 127 Disinfectant

EPA Reg. No. 42182-PA-2

TRANSMITTAL DOCUMENT

1. Name and address of submitter:

Scientific & Regulatory Consultants, Inc.
PO Box 1014
Columbia City, IN 46725

AGENT FOR:
Microban International, Ltd.
11400 Vanstory Drive
Huntersville, NC 28078

2. Regulatory action in support of which this package is submitted:

Review of public health efficacy study protocol within AD; per AD Internal Guidance for the Efficacy Protocol Review Process; applicant initiated; Tier 1
PRIA code A521, $2,363 fee and 3 month review

3. Transmittal date: July 15, 2014

4. Volume 1: Administrative materials

A) Cover letter
B) Application
C) Agent Authorization Letter
D) Proof of PRIA payment made via www.pay.gov
E) Proposed label language
F) Protocol for the Determination of the Residual Self-Sanitizing/Disinfecting Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity

49431601

Company Official: Rhonda Jones

Company Name: Agent for Microban International, Ltd.

Company Contact: Rhonda Jones, Phone: 260-244-6270
E-mail: rjones@srcconsultants.com

Please read instructions on reverse before completing form. Form Approved, OMB No. 2070-0060, Approval expires 5-31-98

United States
Environmental Protection Agency
Washington, DC 20460

☐ Registration
☐ Amendment
☑ Other

OPP Identifier Number

Application for Pesticide - Section I

1. Company/Product Number	2. EPA Product Manager	3. Proposed Classification
42182-PA-2	Jacqueline Hardy	☑ None ☐ Restricted
4. Company/Product (Name)	PM #	
Microban® Firebird 127 Disinfectant	34	

5. Name and Address of Applicant (Include ZIP Code)

Microban International, Ltd.
11400 Vanstory Drive
Huntersville, NC 28078

☐ *Check if this is a new address*

6. **Expedited Review.** In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:

EPA Reg. No. _____

Product Name _____

Section - II

☐ Amendment - Explain below.
☐ Resubmission in response to Agency letter dated _____
☐ Notification - Explain below.
☐ Final printed labels in response to Agency letter dated _____
☐ "Me Too" Application.
☑ Other - Explain below.

Explanation: Use additional page(s) if necessary. (For Section I and Section II.)

Review of public health efficacy study protocol within AD; per AD Internal Guidance for the Efficacy Protocol Review Process; applicant initiated; Tier 1

PRIA Code A521, $2,363 PRIA Fee, 3 month review time. Proof of PRIA payment made via www.pay.gov is attached.

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistant Packaging	Unit Packaging		Water Soluble Packaging		2. Type of Container
☐ Yes ☐ No	☐ Yes ☐ No		☐ Yes ☐ No		☐ Metal ☐ Plastic ☐ Glass ☐ Paper ☐ Other (Specify) _____
***Certification must be submitted**	If "Yes" Unit Packaging wgt.	No. per container	If "Yes" Package wgt	No. per container	

3. Location of Net Contents Information	4. Size(s) Retail Container	5. Location of Label Directions
☐ Label ☐ Container	Various	☐ On Label ☐ On Label accompanying product

6. Manner in Which Label is Affixed to Product: ☐ Lithograph ☐ Paper glued ☐ Stenciled ☐ Other _____

Section - IV

1. Contact Person *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
Rhonda Jones	Agent for Microban International, Ltd.	260-244-6270

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

6. Date Application Received (Stamped)

2. Signature	3. Title
Rhonda Jones	Agent for Microban International, Ltd.
5. Typed Name	5. Date
Rhonda Jones	7/15/2014

Microban®
Microban Products Company

Microban Products Company
11400 Vanstory Drive
Huntersville, NC 28078
Phone (main): 704-875-0806
Fax: 704-875-0810

19 June 2014

Adrienne Turner
U.S. Environmental Protection Agency
Document Processing Desk (NEWCO)
Office of Pesticide Program (7504P)
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001 USA

Dear Adrienne:

SUBJECT: Authorization for Representative/Agent Status
Microban Products Company.

Pursuant to 40 CFR S 152.50(b)(3), Microban International, Ltd. designates Scientific & Regulatory Consultants, Inc. as an authorized agent to act in behalf of Microban International, Ltd. with respect to the new protocol review action being filed as EPA File Symbol 42182-PA-2 as regulated through FIFRA (as amended), that may come before the Agency.

Effective immediately the address of record for this action will be:

Microban Products Company
c/o Scientific & Regulatory Consultants, Inc.
P.O. Box 1014
102 ½ S. Chauncey Street
Columbia City, IN 46725

Representatives of Scientific & Regulatory Consultants, Inc. may be reached via phone at 260-244-6270 or via fax 260-244-6273 if you have any questions or require additional information. Rhonda Jones, President, Scientific & Regulatory Consultants, Inc. will be the designated contact for this action and may be reached at the above telephone or RJones@SRCconsultants.com.

This authorization will remain valid until either Microban Products Company or Scientific & Regulatory Consultants, Inc. gives further notice.

Sincerely,

Neil K. Snyder, Ph.D.
Vice President, Regulatory Affairs
Microban International, Ltd.

Cc: Scientific & Regulatory Consultants, Inc.

Online Payment

Step 3: Confirm Payment

1 | 2 | 3

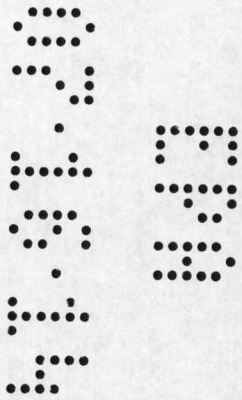
Thank you.
Your transaction has been successfully completed.

Pay.gov Tracking Information

Application Name: PRIA Service Fees
Pay.gov Tracking ID: 25GLRBM1
Agency Tracking ID: 74647973570
Transaction Date and Time: 07/15/2014 09:27 EDT

Payment Summary

Address Information	Account Information	Payment Information
Account Holder Name: Rhonda Jones	**Card Type:** Master Card	**Payment Amount:** $2,363.00
Billing Address: PO 1014	**Card Number:** **********6578	**Transaction Date and Time:** 07/15/2014 09:27 EDT
Billing Address 2:	**Decision Number:**	
City: Columbia City	**Registration Number:** PA-2	
State / Province: IN	**Company Name:** Microban International	
Zip / Postal Code: 46725	**Company Number:** 42182	
Country: USA	**Action Code:** A521	



Note to reviewer: All text in brackets [...] is optional and may or may not be included on a final label. All text in braces {...} is administrative and will not appear on a final label. Portions or all of the label may be bilingual. Claims on this page are optional and may appear on any label panel.

MASTER LABEL

Microban® Firebird 127 Disinfectant

[Microban® 24 Hour Sanitizing Spray]

Active Ingredient:	
Alkyl dimethyl benzyl ammonium chloride	0.16%
Octyl decyl dimethyl ammonium chloride	0.12%
Didecyl dimethyl ammonium chloride	0.06%
Dioctyl dimethyl ammonium chloride	0.06%
Ehanol	70.00%
Other Ingredients:	29.60%
Total	**100%**

KEEP OUT OF REACH OF CHILDREN

WARNING: FLAMMABLE

NET CONTENTS:

EPA Reg. No. 42182- EPA Est. No.

Spray disinfectant for hard, non-porous non-food use surfaces. When used as directed, this product will provide 24 hour self-sanitizing residual antibacterial efficacy on the treated surface. Microban® Firebird 127 Disinfectant will kill 99.9% of bacteria [insert bacteria] on treated surfaces in 30 seconds. Microban® Firebird 127 Disinfectant will provide residual self-sanitizing efficacy against [*Enterobacter aerogenes*] [*Staphylococcus aureus*] [*Pseudomonas aeruginosa*] for up to 24 hours. Residual self-sanitizing efficacy is removed by cleaning and this product must be reapplied to cleaned surfaces to maintain 24-hour sanitization.

May be used in:

Hospitals, clinics and other healthcare [veterinary] [dental] facilities and settings including offices and by emergency responders.

DIRECTIONS FOR USE:

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

To Disinfect: For use on hard, non-porous surfaces. Remove heavy soil from surface prior to applying Microban® Firebird 127 Disinfectant. Hold container 6 – 8 inches from surface and spray until the surface is thoroughly wet. Allow surface to remain wet for the desired contact time as indicated below:

MASTER LABEL

Organism	Contact time
Klebsiella pneumoniae	30 seconds
Pseudomonas aeruginosa	30 seconds
Staphylococcus aureus	30 seconds
Enterobacter aerogenes	30 seconds
Enterococcus faecalis	30 seconds
MRSA	30 seconds
VRE	30 seconds
Aspergillus niger	1 minute

Wipe surface dry with a clean cloth.

Self-Sanitizing Treatment to Provide Residual 24-hour Efficacy Against *Enterobacter aerogenes, Staphylococcus aureus* and *Pseudomonas aeruginosa*: For use on hard, non-porous surfaces. Remove heavy soil and clean surface prior to applying Microban® Firebird 127 Disinfectant. Hold container 6 – 8 inches from surface and spray until the surface is thoroughly wet. Allow surface to remain wet for 5 minutes. Do not rinse treated surface. Wipe surface with a clean cloth. This product can be removed by following the instructions to disinfect, above, by cleaning with a surface cleaner, or with soap and water. After cleaning, repeat the residual self-sanitizing directions to maintain 24 hour sanitization. .

STORAGE AND DISPOSAL:

Do not contaminate water, food, or feed by storage or disposal.

STORAGE: Store in a dry, well-ventilated area at room temperatures. Do not store at temperatures above 49°C/120°F. Avoid all possible sources of ignition, spark or flame.

DISPOSAL: Avoid disposal of unused product. Attempt to use product completely in accordance with the intended use and use directions. Waste resulting from the use of this product can be disposed of on-site or at an approved waste disposal facility.

CONTAINER DISPOSAL: Non-refillable container. Do not reuse or refill empty containers. Dispose of empty container in trash. For disposal of partially filled containers, call your local solid waste agency for disposal instructions. Do not incinerate.

PRECAUTIONARY STATEMENTS:

Hazards to Humans and Domestic Animals

Note to reviewer: All text in brackets [...] is optional and may or may not be included on final label. All text in braces {...} is administrative and will not appear on a final label. Portions or all of the label may be bilingual. Claims on this page are optional and may appear on any label panel.

MASTER LABEL

WARNING: Causes substantial but temporary eye injury or skin irritation. Harmful if swallowed. Do not get in eyes, on skin or on clothing. Do not ingest. Wear goggle or face shield (safety glasses). Wash hands with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse.

FIRST AID:

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 15 minutes and continue rinsing eyes. Get medical attention.

If on skin: Take off contaminated clothing. Wash exposed area with plenty of soap and water and continue to rinse area for at least 15 minutes. If irritation occurs, get medical attention or call a poison control center for further treatment advice.

If ingested: Wash mouth out with water. **DO NOT** induce vomiting unless directed to do so by medical personnel. If conscious, give two glasses of water slowly. Get medical attention immediately. **DO NOT** give anything by mouth if the person is unconscious.

If inhaled: Move person to fresh air. If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth, if possible. Call a poison control center or physician for further treatment advice.

HOTLINE NUMBER (to be provided) In case of accidental exposure call a poison control center or physician. Have the product container or label with you when calling a poison control center or physician, or going for treatment.

PHYSICAL AND CHEMICAL HAZARDS: Flammable. Keep away from heat, sparks, open flames and hot surfaces. Do not smoke when using this product.

ADVISORY STATEMENTS: (if needed)

ANTIBACTERIAL CLAIMS

Disinfectant:

Kills [99.9% of] germs [bacteria] [in 30 seconds]

Hospital disinfectant

Kills [99.9%] of bacteria [MRSA] [VRE] [*Klebsiella pneumoniae*] [*Enterobacter aerogenes*] [*Staphylococcus aureus*] [*Pseudomonas aeruginosa*] [*Enterococcus faecalis*] [in 30 seconds]

Kills [99.9%] of *Aspergillus niger* [1 minute]

Antifungal [spray]

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MASTER LABEL

Antibacterial [spray]

Disinfectant [spray]

Residual Self-Sanitizer:

24 hour sanitizing [disinfectant] spray [with residual benefits]

[Residual] self-sanitizing [disinfecting] spray

24 hour sanitization [disinfection]

[This product] continues to kill 99.9% of bacteria [germs] [MRSA] [VRE] [*Klebsiella pneumoniae*] [*Enterobacter aerogenes*] [*Staphylococcus aureus*] [*Pseudomonas aeruginosa*] even after multiple touches.

Will [continue to] kill [sanitize] [reduce] [inhibit] [suppress] [disinfect] 99.9% of [contaminating] bacteria [germs] [MRSA] [VRE] [*Klebsiella pneumoniae*] [*Enterobacter aerogenes*] [*Staphylococcus aureus*] [*Pseudomonas aeruginosa*] [*Enterococcus faecalis*] that comes in contact with the treated surface for up to 24 hours

[This product] sanitizes [disinfects] for 24 hours.**

[This product] [keeps killing] [continues to kill] 99.9% of bacteria [germs] for 24 hours.**

[This product] will assist in reducing [suppressing] [inhibiting] the spread of [exposure to] bacteria on its surface for [up to] 24 hours.

Organisms

** *Staphylococcus aureus* [Staph], [*Klebsiella pneumonia*] [*Pseudomonas aeruginosa*][Insert Additional Bacteria]

INTRODUCTORY 6-Months CLAIMS – {will only appear on graphic label for the first 6 months the product is on the shelf.}
New[!] [product]

Questions? Comments?
Call: +1 (704) 875-0806
U.S.A. only from 8am - 4:30 pm ET Monday through Friday or online
www.microban.com

Microban International, Ltd.
Global Headquarters
11400 Vanstory Drive

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MASTER LABEL

Huntersville, NC 28078
United States

BATCH CODE:_____

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PROTOCOL

Residual Activity of Dried Chemical Residues on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use

Test Organism(s)

Required Bacterial Strains:

Staphylococcus aureus ATCC 6538
Enterobacter aerogenes ATCC 13048
Pseudomonas aeruginosa ATCC 15442

Additional Bacteria: {*Insert additional strains (e.g. E. coli, MRSA)*}

Product Identity

{*Insert Name of Test Substance*}

{*For Required Strains, insert three lot numbers. For Additional Bacteria, insert two lot numbers. All lots will be manufactured in accordance with EPA LCL Policy (December 2013).*}

Data Requirement

US EPA 40 CFR Part 158
OCSPP 810.2200/810.2300

Study Sponsor

Gina Sloan, Ph.D
Microban Products Company
11400 Vanstory Drive
Huntersville, NC 28078

Performing Laboratory

Antimicrobial Test Laboratories
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Protocol Number

TBD

Prepared by

Antimicrobial Test Laboratories

Date

June 30, 2014 SRC Edits 7-9-14

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Residual Activity of Dried Chemical Residues
on Hard Nonporous Surfaces with Exposure and Wear Activity for Hospital Use

I. Introduction

This document details the materials and procedure used by Antimicrobial Test Laboratories to evaluate the residual activity of a test substance on hard non-porous surfaces based on the US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces and EPA guidance provided February-April 2014 (Continuous Reduction Test Recommendations). This study design may be used to support public health claims. The study is conducted under EPA (40 CFR Part 160) Good Laboratory Practices (GLP) test conditions.

II. Purpose

The purpose of this study is to document the residual activity of the test substance against the test systems (microorganisms) under the test parameters specified in this protocol.

III. Justification for the Selection of Test Systems (Microorganisms)

The United States Environmental Protection Agency (US EPA) requires antimicrobial claims to be supported by relevant test systems (microorganisms). The procedure described was based on US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces and EPA Guidance provided February-April 2014 (Continuous Reduction Test Recommendations). For products which meet the OCSPP 810.2200 requirements for hospital disinfection, this study design may be used to support the addition of a residual disinfection claim for healthcare settings. For products which meet the OCSPP 810.2200 requirements for hospital disinfection and meet the OCSPP 810.2300 requirements for non-food contact sanitizer, this study will also support the addition of a residual sanitization claim for healthcare settings where it meets the contact time and performance requirements stated below.

In accordance with EPA OCSPP 810.2200 and 810.2300, the required bacterial test systems for this study are *S. aureus* (ATCC 6538), *Enterobacter aerogenes* (ATCC 13048) and *Pseudomonas aeruginosa* (ATCC 15442) must also be evaluated. Additional bacteria may be selected for testing (e.g. E. coli, MRSA).

IV. Terms and Conditions

Prior to study initiation, Antimicrobial Test Laboratories must receive the approved and signed protocol and test substances. The proposed Experimental Start Date and Experimental End Date follow and changes to the signed, approved protocol will require amendment.

Proposed Experimental Start Date: TBD

Proposed Experimental End Date: TBD

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Antimicrobial Test Laboratories will repeat studies, free of charge, in the event of unintended protocol non-conformance. The Study Sponsor is responsible for the cost of any repeat study resulting from failure to neutralize the test substance, for the cost of any study performed to confirm the outcome of a previous study, and for ensuring that the study meets their regulatory objectives.

The Study Sponsor must obtain written consent from Antimicrobial Test Laboratories to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

V. Test Substance Identification, Characterization, and Handling

Test Substance Name — {*Insert* ***Test Material Name***}

Lot Number(s): {*Insert* ***Lot No. 1****, and Expiration Date:* ***DD MMM YYYY***}

{*Insert* ***Lot No. 2****, and Expiration Date:* ***DD MMM YYYY***}

{*Insert* ***Lot No. 3*** *for required bacterial strain testing, and Expiration Date:* ***DD MMM YYYY***}

Active Ingredient Concentration — {*Insert the concentration(s). All lots will be manufactured in accordance with EPA LCL Policy (December 2013).*)

Form(s) — {*Insert product form (e.g. Ready to Use Liquid, Spray, Aerosol, Liquid Concentrate)*}

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, and Subpart F [160.105]) is the responsibility of the Study Sponsor. The test substance shall be characterized by the Sponsor prior to the completion of this study. A Certificate of Analysis will be appended to the final report for each test lot.

Test substances are handled as follows:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test substance is shaken or otherwise mixed well immediately prior to use (if applicable).
- The test substance is handled safely in accordance with the chemical risks it may pose, stated in the MSDS or by the Study Sponsor during the course of pre-study communication.

VI. Study Parameters, Incorporated by Reference

Residual Self-Sanitizing / Self-Disinfecting Efficacy

Contact Time – {*Insert exposure period (e.g. 5mins, 10mins, 1 hour).*
For Residual Sanitizer *claims, a 99.9% reduction must be demonstrated in ≤ 5 minutes ± 5 seconds.*
For Residual Disinfection claims, a 99.999% reduction must be demonstrated in ≤ 10 minutes ± 5 seconds.

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Continuous Reduction Period – {*Insert time period (e.g. 12 hours, 24 hours). The following procedure is based on a 24 hour continuous reduction period thus the procedure must be completed in ≥24 hours but no longer than 48 hours. If shorter or longer continuous reduction claims are desired, then the Wear, Abrasion, and Re-inoculation frequencies and the laboratory duration of the procedure must be adjusted ratiometrically. The period must not be less than 4 hours. For example to support a 12 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be reduced by 50% and the study must be completed in ≥12 hours but no longer than 24 hours. For example to support a 48 hour continuous reduction claim, the Wear, Abrasion, and Re-inoculation frequencies described below would be doubled and the study must be completed in ≥48 hours but no longer than 96 hours.*}

Abrasion Control Replicates **- 4 per test microorganism**
Non-Abrasion Control Replicates **– 4 per test microorganism**
Test Surface Replicates **- 4 per test substance, per test microorganism**
Neutralization Controls **- 2 per test substance, per test microorganism inoculum level**

VII. Test Systems (Microorganisms)

Required Bacterial Strains:

Staphylococcus aureus, ATCC 6538
Enterobacter aerogenes ATCC 13048
Pseudomonas aeruginosa ATCC 15442

Additional Bacteria: {*Insert additional strains (e.g. E. coli, MRSA)*}

Required bacteria will be tested on 3 lots per organism. Additional bacteria will be tested on 2 lots per organism.

VIII. Materials

Reagents, Media, and Supplies

- Sufficient quantity of test substance(s).
- Sufficient quantity of 1 x 1 inch non-frosted glass slides (carriers) free of visible damage.
- Sufficient quantity of sterile Whatman No. 2 filter paper.
- Sufficient quantity of clean, sterile Petri plates.
- Sufficient quantity of sterile Reverse Osmosis (R/O) water.
- Sufficient volume of sterile Fetal Bovine Serum (FBS).
- Sufficient volume of Triton X-100.
- Sufficient sterile tubes containing sterile Reverse Osmosis (R/O) water, for dilution of microbial suspensions.

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- Bunsen burner, microbiological incinerator, or micro-torch as appropriate to ensure rapid and complete flame-sterilization of forceps and/or loops.
- Sufficient quantity of micropipettes and appropriately sized sterile micropipette tips.
- Automatic pipettor (PipetAid or similar) and various sizes of sterile serological pipettes.
- Sufficient quantity of sterile 50 ml centrifuge tubes.
- Sufficient quantity of foam liners (Manufacturer: ITX Texwipe, Type: TX 704) and sterile, approximately 2-inch-wide, strips of cotton cloth (Manufacturer: ITW Texwipe, Type: Texwipe TX 312).
- Gardco Washability and Wear Tester (Model D10V, Cat. #WA-2153, Paul N. Gardner Co., Inc., Pompano Beach, FL).Appropriate Spray bottle and Preval sprayer and bottle(s).
- Forceps.
- Vortex mixer.
- Sonicating water bath.
- Orbital shaker.
- Inoculating loops.
- Appropriate volume of 95% and absolute ethanol.
- Certified digital timer.
- Certified satellite clock.
- Water bath.
- Calibrated hygrometer.
- Calibrated thermometer.
- Calibrated laboratory balance, with an accuracy of at least 0.001g.
- Pure culture of each test system (microorganism).
- Sufficient quantity of test tubes containing 10 ml sterile AOAC nutrient broth.
- Sufficient quantity of sterile Tryptic Soy agar (TSA), tempered to 48±2°C.
- Sufficient quantity of test tubes containing 30 ml of sterile Dey/Engley Neutralizer Broth (or other as deemed appropriate for neutralization of the test substance).
- Sufficient number of test tube racks.
- Incubator capable of sustaining temperatures of 35±2°C.
- Incubator capable of sustaining temperatures of 30±2°C.

IX. Procedure: An overview of the testing procedure is provided in Table 1.

Preparation of Carriers

- 1" x 1" glass carriers are visually screened prior to use in the study and flawed carriers are discarded.
- Prior to the test, carriers are rinsed with 95% ethanol to remove oil and film.
- Carriers are thoroughly rinsed using multiple tap-water rinses followed by a double reverse osmosis water rinse, then allowed to air dry.
- Carriers are decontaminated by autoclave sterilization and then aseptically transferred to sterile Petri plates lined with 1-2 layers of Whatman No. 2 filter paper.

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Preparation of Abrasion Control Substance

- A 0.01% Triton X-100 solution is prepared in sterile reverse osmosis water.
- The prepared control solution is sprayed from an ethanol-sanitized Preval spray bottle and used to treat control carriers. The same spray time and distance will be used as described for the test lots.

Preparation of Test Cultures

- An isolated colony is transferred from the most recent monthly working stock transfer to 10 ml AOAC Nutrient Broth and incubated at 30 ± 2° C for *E. aerogenes* and 35 ± 2° C for *S. aureus* or *K. pneumoniae*. A minimum of 3 consecutive daily transfers are made by transferring a loopful of the previous transfer into 10ml sterile Nutrient Broth and prior to inoculating the Initial Inoculation Culture, Reinoculation Culture or Final Test Culture.
- The Initial Inoculation Culture (transfer ≥4) is incubated for 48 - 54 hours at 30 ± 2° C for *E. aerogenes* and 35 ± 2° C for *S. aureus, K. pneumoniae* or *P. aeruginosa.* The culture is vortexed for 3 - 4 seconds and allowed to sit for 15 ± 1 minutes. The culture is diluted in sterile R/O water supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^6 CFU/carrier. The final FBS supplemented suspension is vortexed for 3- 4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.
  NOTE: For *P. aeruginosa*, the pellicle from the inoculum culture must be removed prior to vortexing and before testing is initiated, this is done by utilizing a vacuum apparatus to remove and dispose of the pellicle. Any disruption of the pellicle resulting in dropping or breaking up of the pellicle in the culture before or during its removal renders that culture unusable in the residual test.

- The Reinoculation Culture (transfer ≥4) is incubated for 18 - 24 hours at 30 ± 2° C for *E. aerogenes* and 35 ± 2° C for *S. aureus , K. pneumoniae* or *P. aeruginosa.* The culture is vortexed for 3 - 4 seconds and allowed to sit for 15 ± 1 minutes. The culture is then diluted in sterile R/O water and is supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^4 CFU/carrier. The final FBS supplemented suspension is vortexed for 3- 4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.
- The Final Test Culture (transfer ≥4) is incubated for 18 - 24 hours at 30 ± 2° C for *E. aerogenes* and 35 ± 2° C for *S. aureus, K. pneumoniae* or *P. aeruginosa.* The culture is vortexed for 3 - 4 seconds and allowed to sit for 15 ± 1 minutes. The culture is diluted in sterile R/O water and supplemented with fetal bovine serum to yield a 5% (v/v) final FBS concentration to obtain a final target concentration of 1 x 10^6 CFU/carrier. The final FBS supplemented suspension is vortexed for 3- 4 seconds and allowed to stand at room temperature for a minimum of 15 minutes prior to use.

Carrier Inoculation with "Initial Inoculation Culture"

- 0.010ml of the "Initial Inoculation Culture" is spread to within 1/8 inch of the surface edge of each test and control carrier with a micropipette tip bent to approximately 45° angle.
- All inoculated carriers are dried uncovered at 35 ± 2°C for 30-35 minutes, or until visibly dry.

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Exposure of Abrasion and Non-Abrasion Control Carriers to Control Substance

- Abrasion and Non-Abrasion Control Carriers (a subset of all inoculated carriers) are treated with sterile 0.01% Triton X-100 solution by treating in the same manner as test carriers.
- The solution on the carriers after treatment is allowed to dry at room temperature and 45-55% relative humidity with lids ajar for 30 minutes, or until visually dry.

Exposure of Test Carriers to Test Substance

- Four test carriers (per lot, per microorganism) are treated by spray application with the test substance. Each carrier is treated according to the study sponsor's instructions.
- After treatment, the test substance on the carriers is allowed to dry at room temperature and 45-55% relative humidity with the lids ajar for up to 1 hour such that the first Wear cycle begins no later than no longer than 1 hour after treatment of the carrier.

Abrasions and Re-inoculations

- Test Carriers and Abrasion Control Carriers undergo a wear and re-inoculation regimen including a series of at least 12 wear cycles and 12 re-inoculation cycles to support a 24 hour continuous reduction claim. The Non-Abrasion Control Carriers do not undergo the wear cycling. The number, duration, and order of abrasions may be ratiometrically modified by the Study Sponsor to support the desired claim as described above. This step is performed at room temperature. The table below summarizes the manipulations of all carriers in the study.
- Abrasions are conducted between 45-55% relative humidity (RH). Temperature and room humidity measurements are taken and recorded periodically throughout the abrasion process.
- The weight of the fully assembled abrasion boats are recorded prior to initiation of the wear and re-inoculation regimen and must equal 1084 ± 1.0g
- The abrasion tester is set to a speed of 2.25 to 2.5 for a total surface contact time of approximately 8-10 seconds, for one complete abrasion cycle. Each abrasion cycle in this test equals four (4) passes, one pass to the left and one return pass to the right followed by another pass to the left and another return pass to the right.
- All surfaces in contact with carriers on the abrasion tester are decontaminated with ethanol and allowed to dry completely between each set of surface wears to prevent carryover contamination.
- The foam liner and cotton cloths on the abrasion tester are replaced between each set of abrasion.
- After each complete set of abrasions are conducted (all control and test carriers abraded), the carriers are allowed to sit undisturbed for at least 15 minutes.
- Control and test carriers are then re-inoculated with 0.010ml of the re-inoculation culture and spread within 1/8 inch of the surface edge, and then allowed to dry at ambient temperature for a minimum of 30 minutes or until completely dry prior to initiation of the next set of abrasions.

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- Cotton cloths used as part of wet abrasions are prepared individually prior to each wet abrasion cycle by spraying the cloth with sterile reverse osmosis water using a sanitized Preval sprayer, from a distance of 75±1cm for no more than 1 second and used immediately.

Table 1. Example of procedure timeline and target concentrations for a 24hr Residual Claim

Procedure Timeline (Hours)	**Abrasion/Re-Inoculation Procedure**	**Target CFU/carrier**
0	Initial inoculation of Test and Control Carriers	10^6
1	Test Substance Application and Drying	
1->24	Dry Abrasion (Wear #1)	10^4 with each reinoculation
	Reinoculation(1)*	
	Wet Abrasion (Wear #2)	
	Reinoculation(2)*	
	Dry Abrasion (Wear #3)	
	Reinoculation(3)*	
	Wet Abrasion (Wear #4)	
	Reinoculation(4)*	
	Dry Abrasion (Wear #5)	
	Reinoculation(5)*	
	Wet Abrasion (Wear #6)	
	Reinoculation(6)*	
	Dry Abrasion (Wear #7)	
	Reinoculation(7)*	
	Wet Abrasion (Wear #8)	
	Reinoculation(8)*	
	Dry Abrasion (Wear #9)	
	Reinoculation(9)*	
	Wet Abrasion (Wear #10)	
	Reinoculation(10)*	
	Dry Abrasion (Wear #11)	
	Reinoculation(11)*	
	Wet Abrasion (Wear #12)	
	Reinoculation(12)*	
≥24 - 48	Determination of Residual Activity	10^6

Test and Control Carrier Wear and Re-inoculation Regimen

Determination of Residual Activity

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- Residual activity is determined for all Test and Abrasion Control carriers after the last of the 12 wear and 12 re-inoculation cycles, and at least 24 hours but not more than 48 hours after the product application.
- Carriers are sequentially inoculated with 0.010 ml of the "Final Test Culture" at an appropriate interval, spreading the inoculum to within 1/8 inch of the edge, and then letting stand for the Sponsor requested contact time (see Study Parameters, Page 3). Start and stop times are recorded.
- After the contact time has elapsed, carriers are aseptically transferred into labeled tubes containing 30 ml of neutralizer broth.
- Samples are sonicated for 20 ± 2 seconds in a sonicating waterbath. The samples are then sufficiently vortexed.
- The Abrasion Control samples are serially diluted in 0.900ml of sterile reverse osmosis water, or other suitable diluent. All samples are plated in duplicate and within approximately 30 minutes of their transfer to the neutralizer broth.
- The test carriers are serially diluted in 0.900ml of sterile reverse osmosis water, or other suitable diluent. The appropriate dilutions of the test carrier are prepared and plated in duplicate using pour plate techniques. All samples are plated within approximately 30 minutes of their transfer to the neutralizer broth.
- Plates are incubated at incubated at 30 ± 2° C for *E. aerogenes* and 35 ± 2° C for *S. aureus* and *P. aeruginosa* for 48 – 54 hours.

Inoculum Concentration Determinations

- The concentrations (CFU/ml) of the Initial Inoculation Culture, Reinoculation Culture(s), and the Final Test Culture are determined by serial dilution in sterile reverse osmosis water and plating in duplicate.
- Plates of the test microorganisms are incubated at 30 ± 2° or 35 ± 2° C, as appropriate for the microorganism, for 48 - 54 hours.

Recording of Environmental Conditions

- The temperature and humidity of the testing area are recorded at appropriate intervals throughout testing.

Calculations

- The Geometric Mean of the number of microorganisms surviving on four control surfaces or four test surfaces = Antilog (Log_{10} X1 + Log_{10} X2 + Log_{10} X3 + Log_{10} X4)/4, where X equals the number of microorganisms surviving per carrier.

- The Percent Reduction of microorganisms surviving on test surfaces over microorganisms surviving on parallel Abrasion Control surfaces = [(Geometric Mean of Abrasion Control surfaces – Geometric Mean of test surfaces)/Geometric Mean of control surfaces] x 100

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X. Experimental Controls

Neutralization Validation Control

- For each organism tested, duplicate test surfaces are treated with the test product according to study sponsor directions along with duplicate test surfaces treated with control solution.
- Neutralization Validation test surfaces are treated and dried on Day 1 (i.e. in parallel with test and control surfaces that will undergo wear and re-inoculation regimen) and allowed to sit undisturbed for the duration of the study.
- Treated and control test surfaces are aseptically transferred to 30 ml neutralization broth during the "Determination of Residual Activity" portion of the study.
- Neutralized carriers are inoculated with 0.100 ml of a dilute suspension of Final Test Culture, obtained via serial dilution in PBS, to yield ≤300 CFU/ml.
- A separate 30 ml neutralization broth tube is inoculated with 0.100 ml of the same dilute suspension and serves as the inoculum control.
- Neutralized samples are sufficiently vortexed and are held for 5 ± 1 minutes.
- After the specified hold time, a 1 ml aliquot is removed from each tube and pour plated in duplicate to determine viable CFU/ml.
- The effectiveness of the chosen neutralizer is validated if the counts recovered from the treated carriers are within 0.5 $\log_{10}$ of the control carriers.

Inoculated Carrier Viability Control

- An additional 2 carriers per microorganism are inoculated with the initial inoculation culture and dried along with other test and control carriers and for each test microorganism. After the dry time the carriers are harvested and vortexed for 10 seconds ± 2 seconds. The tubes are incubated along with the plates at 30 ± 2° or 35 ± 2° C as appropriate for the microorganism for 48 - 54 hours. Presence of growth is determined by a change of color or turbidity of the neutralization broth after incubation.

Initial Inoculation Carrier Controls

- Two sterile carriers are inoculated with the Initial Inoculation Culture and recovered immediately..
- Carriers are harvested and enumerated following the steps detailed in the "Determination of Residual Activity" section of the protocol.

Reinoculation Carrier Control

- Two sterile carriers are inoculated upon initial use of each prepared Re-inoculation Culture and recovered immediately.
- Carriers are harvested prior to initiating abrasions and enumerated following the steps detailed in "Determination of Residual Activity" section.

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Purity Control

- An isolation streak is performed for each test culture to verify culture purity.

"Soil" Sterility Control

- 0.100ml of "soil" is plated to appropriate agar for sterility confirmation and incubated alongside the test to verify sterility. Presence of growth is determined by change of color or turbidity of the neutralization broth in the tube.

Media/Diluent Sterility Control

- A plate or aliquot of all media (growth and enumeration media) and diluents is incubated alongside the test to verify media sterility. Presence of growth is determined by change of color or turbidity of the neutralization broth in the tube.

Carrier Sterility Control

- One sterile, uninoculated, untreated carrier is harvested in 30ml neutralization broth. The tube is incubated alongside the test. Presence of growth is determined by change of color or turbidity of the neutralization broth in the tube.

Initial Inoculum Viability Control

- An additional 2 carriers per microorganism are inoculated with the initial inoculation culture and dried along with other test and control carriers and for each test microorganism. After the dry time the carriers are harvested in 30 ml neutralization broth and the tubes are incubated along with the plates at 30 ± 2° or 35 ± 2° C, as appropriate for the microorganism, for 48 - 54 hours. The presence of growth is determined by a change of color or turbidity of the neutralization broth after incubation.

XI. Incubation of Plates and Controls

- All enumeration plates and controls are incubated at 30 ± 2° or 35 ± 2° C, as appropriate for the microorganism, for 48 - 54 hours.

XII. Calculations and Statistical Analysis, and Control of Bias

- For quantitative determinations, see calculations in the respective "Calculations for Test" section, above.
- Statistics and control of bias is not applicable to this *in vitro* study.

XIII. Success Criteria

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- The experimental success (controls) criteria follow:
  1. In the Neutralization Control, test substance treated carrier counts must be within 0.50 $\log_{10}$ of the control treated carrier counts.
  2. The media sterility controls are negative for growth.
  3. The purity "isolation streaks" demonstrate a pure culture of test microorganism as evidenced by colony morphology.
  4. The carrier sterility controls are negative for growth.
  5. The soil sterility control is negative for growth.
  6. The Initial Inoculation Carrier Control must have a minimum of 1×10^6 CFU/carrier.
  7. The Re-Inoculation Carrier Control carriers must have a minimum of 1×10^4 CFU/carrier.
  8. The Final Abrasion Control must have a minimum of 1×10^6 CFU/carrier.

- Test substance performance criterion for public health claims:
- To be defined as a residual disinfectant for healthcare use, the test product must:
  - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
  - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 5 $\log_{10}$ or 99.999% at a contact time of ≤ 10 minutes.
- To be defined as a residual sanitizer for healthcare use, the test product must:
  - meet the OCSPP 810.2200 requirements for a hospital disinfectant, and
  - meet the OCSPP 810.2300 requirements for a non-food contact sanitizer, and
  - in this study reduce the total number of organisms on a hard, nonporous, inanimate surface over the parallel Abrasion Control by at least 3 $\log_{10}$ or 99.9% at a contact time of ≤ 5 minutes.

XIV. Reporting

- The report will include, but is not limited to, identification of the sample, date received, dates on which the test was initiated and completed, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185. A draft final report will be provided to the Sponsor for review prior to finalization.

XV. Data and Sample Retention

- The study report, and corresponding data will be held in the archives of Antimicrobial Test Laboratories for at least 2 years after the date of the final report. After 2 years, documentation may be returned to the Study Sponsor for archiving.
- The test substance may be returned to the Study Sponsor at Sponsor's request and expense within 30 days of study completion. If the Study Sponsor does not request return of the sample, it will be

This document will not be made publicly available until the subject protocol is approved by EPA and EPA grants a claim based upon the protocol.

destroyed >30 days after study completion. Archiving of test substances is the responsibility of the Sponsor.

XVI. Quality Control

- The study will be conducted in accordance with Antimicrobial Test Laboratories' Quality Management System and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XVII. References

- US EPA Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces. Protocol number 01-1A.

XVIII. Protocol Approval

"I, the Study Sponsor, have read and understand the study protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test(s) to be completed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR 160. I have also read, understand and agree to the terms and conditions listed in the protocol."

Study Sponsor/Representative Signature Approving Protocol

_____ _____

Study Sponsor Date

_____ _____

Study Director Date